09/937779

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
3377/99 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/02701	28/03/2000	01/04/1999
International Patent Classification (IPC) or national classification and IPC C12N15/54		
Applicant BASF PLANT SCIENCE GmbH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 12 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

CORRECTED VERSION

Date of submission of the demand	Date of completion of this report
11/10/2000	25.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Page, M Telephone No. +49 89 2399 7322

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/02701

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-32 as originally filed

Claims, No.:

1-22 as received on 20/04/2001 with letter of 18/04/2001

Drawings, sheets:

1/6-6/6 as originally filed

Sequence listing part of the description, pages:

1-45 (SEQ ID NOs. 1-15), as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 22 (partially).

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☒ the claims, or said claims Nos. 22 (partially) are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	9-19, 21, 22 (all partially)
	No:	Claims	1-19, 21, 22 (all partially)
Inventive step (IS)	Yes:	Claims	9-19, 21, 22 (all partially)
	No:	Claims	1-19, 21, 22 (all partially)
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The application concerns the provision of a yeast and plant polypeptide and polynucleotide sequences allegedly corresponding to diacylglycerol acyltransferases. Function is shown for Saccharomyces cerevisiae sequences, but neither the function nor any structural relationship to the Saccharomyces sequences making such a function plausible are demonstrated for the other full-length and partial sequences.

Re Item II
Priority

After considering the priority document, the documents cited "P, X" in the search report are not considered relevant for the examination of novelty and inventive step.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 18 (claim 22 as originally filed) seeks protection for cells or organisms with altered PDAT activity, "wherein the altered PDAT activity is characterized by an alteration in gene expression, catalytic activity and/or regulation of activity of the enzyme". No reference could be found in the description for alterations to the catalytic activity or regulation of PDAT activity and claim 18 (partially) is therefore considered to lack meaningful support from the description. The claim has only been examined with respect to alterations in gene expression.

Re Item IV
Lack of Unity of Invention

An international application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept. Unity of invention is fulfilled only when there is a technical relationship between the inventions involving one or more of the same or corresponding special technical features. Special technical features are such features that define the contribution of the claimed

invention over the prior art.

The identified 8 inventions relate to a group of sequences with the claimed technical feature of being diacylglycerol acyltransferases as the sole common link. However, this feature cannot be considered to constitute a special technical feature because it does not define a contribution over the prior art: SEQ ID NOs. 2, 3, 9, 16, 20 and 22 have all been previously disclosed in their entirety (D1, D2 and D3).
Although the prior art does not disclose the function of the encoded enzymes, they do disclose the nucleic acid and polypeptide sequences of the respective claimed SEQ ID NOs. The encoded enzyme will have the activity claimed in claim 1, regardless of whether or not this is disclosed in the prior art.
The application therefore does not meet the requirements of Rule 13.2 PCT in that there is no common special technical feature linking the 8 inventions of the application, these being:

Invention I **Claims 5, 6, 8-22 (all partially) and 1-3 (completely) (formerly claims 1, 3, 6, 7, 9, 11-27 (all partially) 2 and 4 (completely))**

Enzymes catalysing the acyl-CoA-independent transfer of fatty acids to diacylglycerol in the production of triacyglycerol from *Saccharomyces cerevisiae* and corresponding to polypeptides with SEQ ID NOs. 2, 16, 20 and 22, encoded by polynucleotides SEQ ID NOs. 1, 19 and 21, fragments, derivatives, alleles, homologs and isoenzymes, the corresponding polynucleotide sequences, portions, derivates, alleles and homologs of the polynucleotide sequence, expression vectors, transgenic cells and organisms, processes for the production of triacylglycerol using such cells/organisms, the product of such a process and the use of the enzymes and polynucleotides in such processes.

Invention II **Claims 4-6 and 8-22 (all partially) (formerly claims 1, 3, 5-9 and 11-27 (all partially))**

As invention I with SEQ ID NOs. 3, 13 and 23 from *Schizosaccharomyces pombe*.

Invention III **Claims 4-22 (all partially) (formerly claims 1, 3 and 5-27 (all partially))**

As invention I with SEQ ID NOs. 4-6, 18, 24, 25 from *Arabidopsis thaliana*.

Invention IV **Claims 4, 5 and 7-22 (all partially) (formerly claims 1, 3 and 5-27 (all partially))**

As invention I with SEQ ID NOs. 7, 8, 26 and 27 from *Zea mays*.

Invention V **Claims 5 and 7-22 (all partially) (formerly claims 1, 3, 6-8 and 10-27 (all partially))**

As invention I with SEQ ID NOs. 9 and 28 from *Neurospora crassa*.

Invention VI **Claims 4-6 and 8-22 (all partially) (formerly claims 1, 3, 5-9 and 11-27 (all partially))**

As invention I with SEQ ID NOs. 10, 14, 17 and 29 from *Arabidopsis thaliana*.

Invention VII **Claims 4-6 and 8-22 (all partially) (formerly claims 1, 3, 5-9 and 11-27 (all partially))**

As invention I with SEQ ID NOs. 11, 15 and 30 from *Arabidopsis thaliana*.

Invention VIII **Claims 5 and 7-22 (all partially) (formerly claims 1, 3 and 5-27 (all partially))**

As invention I with SEQ ID NOs. 12 and 31 from *Lycopersicon esculentum*.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) **Reference is made to the following documents:**

D1: PETER VERHASSELT ET AL.: 'Twelve open reading frames revealed in the 23.6kb segment flanking the centromere on the Saccharomyces cerevisiae chromosome XIV right arm' YEAST, vol. 10, no. 7, July 1994 (1994-07), pages 1355-1361, XP002112572 -& Swissprot Database Entry Yn84_Yeast Accession number P40345; 1 February 1995 XP002112574

D2: DATABASE EMBL [Online] Database Entry SPBC776, 21 January 1999 (1999-01-21) LYNE M. ET AL.: 'S. pombe chromosome II cosmid c776' Database accession no. AL035263 XP002150203

D3: DATABASE EMBL [Online] Database Entry AI398644, 10 February 1999

(1999-02-10) XP002150204 & MARY ANNE NELSON ET AL.: 'Expressed sequences from conidial, mycelial, and sexual stages of Neurospora crassa ' FUNGAL GENETICS AND BIOLOGY, vol. 21, 1997, pages 348-363, XP000952173

D4: KEITH STOBART ET AL.: 'Triacylglycerols are synthesized and utilized by transacylation reactions in microsomal preparations of developing safflower (Carthamus tinctorius L.) seeds' PLANTA, vol. 203, no. 1, 1997, pages 58-66, XP002112573

D5: WO 98 55631 A (CALGENE LLC) 10 December 1998 (1998-12-10)

2) **Novelty - Art.33(1) and (2) PCT**:

Invention I Claims 5, 6, 8 (all partially) and 1-3 (completely)

Claims 5, 6, 8 (partially), and 1-3 (completely) lack novelty in light of the sequence with the accession number P40345 provided by D1 (identified therein as N2042) which, according to the description of the present application, encodes an acyl-CoA-independent acyltransferase. Although D1 does not disclose the function of the encoded enzyme, a polynucleotide or polypeptide sequence is not rendered novel by the discovery of its function. The disclosed sequence is 100% identical to SEQ ID NO. 2 over the whole length of the protein.

Inventions II-VIII Claims 4-8 (all partially)

Claims 4-8 (partially) lack novelty in light of the sequences provided by D1, D2 and D3 which, according to the description, are polypeptides and polynucleotides corresponding to phospholipid:diacylglycerol acyltransferases. As stated previously, Identifying the function of known polypeptides does not render the polypeptides novel.

The description, for example, defines a "functional fragment" on page 4 lines 30-32 as being "*any* polypeptide sequence which shows specific enzyme activity of a *PDAT*" The enzyme N2042 disclosed in D1 clearly possesses such activity and thus the claims lack novelty.

Similarly, allelic variants are understood to be "any different nucleotide sequence which encodes a polypeptide with a functionally different function" and having an undisclosed number of substitutions, additions or deletions (page 5 lines 28). Again,

the protein of D1 clearly fulfills these requirements.
The definition provided on page 6 lines 17 and 18 for the term "isoenzyme" meets the same objections.
Furthermore, the definition in the description for the term "portion" is meant to include any nucleotide sequence which shows specific activity of a PDAT" (page 5 lines 7-17). The term includes within its scope the polynucleotide sequences AI398644 of D3 for example.

Inventions I-VIII Claims 9-22
Claims 9-19, 21 and 22 (partially) appear to be novel in light of the cited prior art. although polynucleotide and polypeptide sequences according to the claimed invention have been disclosed (e.g. D1 sequence N2042, D2 sequence O94680, D3), these documents do not disclose gene constructs, vectors, transgenic cells ro the use of such in the production of triacylglycerol.

Claim 20 (partially) lacks novelty in light of D4, which discloses triacylglycerol made with an acyl-Co-A independent acyltransferase (D4 page 59 left-hand column paragraph 1). Even if the claim were restricted to triacylglycerol made using novel subject matter, the Applicant would need to show how this product differs from previously disclosed subject matter, as a product is not rendered novel by a new method for making it.

3) **Inventive Step - Art.33(1) and (3) PCT**:

The following comments on inventive step are confined to subject matter which could be acknowledged as being novel, or for which novelty could potentially be restored as outlined supra.

Invention I Claims 9-19, 21 and 22 (all partially)
The closest prior art is document D5, which discloses a the polypeptide and polynucleotide sequences for an acyl-Co-A dependent plant diacylglycerol acyltransferase as well as the use of the sequences in engineering plants with altered triacylglycerol content (D5 page 3 line 22 to page 5 line 20).

In the light of the prior art, the technical problem can be regarded as the provision of further polynucleotide and polypeptide sequences encoding enzymes that can alter the triacylglycerol content of cells or organisms expressing them.

Claims 9-19, 21 and 22 appear to be inventive in light of the cited prior art, which does not disclose the enzyme activity of SEQ ID NO. 2. There is therefore no motivation to combine the teaching of D5 with that of D1 disclosing the sequence N2042.

Inventions II-VIII 9-19, 21 and 22 (all partially)
Again, the closest prior art is document D5, which discloses a the polypeptide and polynucleotide sequences for an acyl-Co-A dependent plant diacylglycerol acyltransferase as well as the use of the sequences in engineering plants with altered triacylglycerol content (D5 page 3 line 22 to page 5 line 20).

In the light of the prior art, the technical problem can be regarded as the provision of further polynucleotide and polypeptide sequences encoding enzymes that can alter the triacylglycerol content of cells or organisms expressing them.

It cannot be seen how inventive step can be recognised for **claims 9-19, 21 and 22**. Although function has been demonstrated for the enzyme encoded by SEQ ID NO. 1, no such function has been demonstrated for the sequences from other species, nor has the Applicant shown that there is a structural relationship between the sequences of Invention I and those of Inventions II-VIII that would make such a function plausible. This is true for the full-length sequences as well as the partial sequences disclosed in the application.

Re Item VII
Certain defects in the international application

a) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D5 are not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

a) Several of the SEQ ID NOs. appear to be identical duplicates of each other, resulting in a lack of conciseness as required by Article 6 PCT. The unnecessary duplicates should be removed.

Claims

1. An enzyme, designated as phospholipid:diacylglycerol acyltransferase (PDAT), catalysing in an acyl-CoA-independent reaction the transfer of fatty acids from phospholipids to diacylglycerol in the biosynthetic pathway for the production of triacylglycerol and comprising an amino acid sequence as set forth in SEQ ID No. 2 or a functional fragment, derivate, allele, homolog or isoenzyme thereof.

2. An enzyme according to claim 1 comprising an amino acid sequence encoded through a nucleotide sequence as set forth in SEQ ID No. 1 or a homologous nucleotide sequence which is at least about 40% identical to a nucleotide sequence of SEQ ID NO. 1.

3. An enzyme according to claims 1 or 2, comprising an amino acid sequence as set forth in SEQ ID No. 16, 20 or 22 or a functional fragment, derivate, allele, homolog or isoenzyme thereof.

4. An enzyme according to claims 1 to 3, comprising an amino acid sequence selected from the group consisting of sequences as set forth in SEQ ID No. 6, 8, 13, 14, 15, 17, 18, 25 or 27 or a functional fragment, derivate, allele, homolog or isoenzyme thereof.

5. An enzyme according to claims 1 to 4, comprising an amino acid sequence encoded through a nucleotide sequence, a portion, derivate, allele or homolog thereof selected from the group consisting of sequences as set forth in SEQ ID No. 1, 3, 4, 5, 7, 9, 10, 11, 12, 19, 21, 23, 24, 25, 26, 28, 29, 30 or

31 or a functional fragment, derivate, allele, homolog or isoenzyme of the enzyme encoding amino acid sequence.

6. A nucleotide sequence according to claims 2 or 5, selected from the group consisting of sequences as set forth in SEQ ID No. 1, 3, 4, 10, 11, 19, 21, 23, 24, 29 or 30 or a portion, derivate, allele or homolog thereof.

7. A partial nucleotide sequence corresponding to a fullength nucleotide sequence according to claims 2, 5 or 6, selected from the group consisting of sequences as set forth in SEQ ID No. 5, 7, 9, 12, 25, 26, 28 or 31 or a portion, derivate, allele or homolog thereof.

8. A nucleotide sequence according to claims 2 or 5 to 7, comprising a nucleotide sequence which is at least 40% identical to a nucleotide sequence selected form the group consisting of those sequences set forth in SEQ ID No. 1, 3, 4, 5, 7, 9, 10, 11, 12, 19, 21, 23, 24, 25, 26, 28, 29, 30 or 31.

9. A gene construct comprising a nucleotide sequence according to claims 2 or 5 to 8 operably linked to a heterologous nucleic acid.

10. A vector comprising a nucleotide sequence according to claims 2 or 5 to 8 or a gene construct according to claim 9.

11. A vector according to claim 10, which is an expression vector.

12. A vector according to claims 10 or 11, further comprising a selectable marker gene and/or nucleotide sequences for the replication in a host cell or the integration into the genome of the host cell.

13. A transgenic cell or organism containing a nucleotide sequence according to claims 2 or 5 to 8 and/or a gene construct according to claim 9 and/or a vector according to claims 10 to 12.

14. A transgenic cell or organism according to claim 13 which is an eucaryotic cell or organism.

15. A transgenic cell or organism according to claims 12 or 13 which is a yeast cell or a plant cell or a plant.

16. A transgenic cell or organism according to claims 12 to 15 having an altered biosynthetic pathway for the production of triacylglycerol, characterized by the prevention of accumulation of undesirable fatty acids, which are harmful if present in high amounts in membrane lipids.

17. A transgenic cell or organism according to claims 12 to 16 having an altered, increased oil content.

18. A transgenic cell or organism according to claims 12 to 17 wherein the activity of PDAT is altered, characterized by an alteration in gene expression, catalytic activity and/or regulation of activity of the enzyme.

19. A process for the production of triacylglycerol, comprising growing a transgenic cell or organism according to claims 13 to 18 under conditions whereby the said nucleotide sequence according to claims 2 or 5 to 8 is expressed.

20. Triacylglycerols produced by a process according to claim 19.

21. Use of a nucleotide sequence according to claims 2 or 5 to 8 and/or an enzyme according to claims 1, 3 or 4 for the production of triacylglycerol and/or triacylglycerols with uncommon fatty acids, comprising medium chain fatty acids, hydroxylated fatty acids, epoxygenated fatty acids and acetylenic fatty acids.

22. Use of a nucleotide sequence according to claims 2 or 5 to 8 and/or an enzyme according to claims 1, 3 or 4 for the transformation of any cell or organism in order to be expressed in this cell or organism and result in an altered, preferably increased oil content of this cell or organism.